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The Germination of Seeds with Coats Impermeable to Water.

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THE GERMINATION OF SEEDS WITH COATS IMPERMEABLE TO WATER

BY

EDWARD GEORGE LAUTERBACH
B. S. University of Illinois, 1915

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF SCIENCE

IN BOTANY

IN

THE GRADUATE SCHOOL
OF THE
UNIVERSITY OF ILLINOIS
1917

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THE GRADUATE SCHOOL

May 26, 1917

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Edward George Lauterbach

ENTITLED The Germination of Seeds with Coats Impermeable to Water

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science in Botany

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Recommendation concurred in:*

Committee
on
Final Examination*

*Required for doctor's degree but not for master's.

4 Feb. 18 Cilley

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I Introduction

Germination tests, so often made to determine the germinating power of seeds, are frequently misleading as to the true viability of the seeds. Of the numerous factors limiting germination, that of impermeability of the seed coats is by far the one most worthy of attention. Since the possession of an impermeable coat is common to a large number of horticultural and agricultural seeds, and germination during the first or subsequent seasons is thereby impossible, a considerable loss to the farmer is apparent. In some otherwise very desirable varieties, the quantity of seed sown necessary to get a "stand" makes their use well-nigh prohibitive because of expense. Harrington (4) found in commercial lots of sweet clover seed that the proportion of hard (impermeable) seeds varied from less than ten to more than ninety percent. He also found a large percent of these hard seeds in hand-hulled samples of red clover, alsike clover, and white clover, while in machine-hulled samples of the same varieties the percent was usually comparatively small but varied with the type of clover huller used. At a price of $10 per bushel, the loss to the farmers of the State of Illinois on alfalfa and clovers probably amounts to more than $181,500 annually. Numerous attempts have been made to overcome this loss by mechanical abrasion, or otherwise making the seed coat permeable to water. Among the more common methods employed, the following may be mentioned: first, a hot water treatment which is merely soaking the seeds in hot water for a shorter or longer period of time; second, a mechanical treatment by which the seed coat is scratched or abraded; and third, treatment with acids or other chemical agents by which the

nature of the seed coat is altered and made permeable to water.

The hot water treatment was probably earliest used by practical seedsmen, tho not first investigated scientifically. It was recommended in 1893 by Bruyning (2), later by Wernicke (15) in 1895, by Jarzymouski (6) in 1905, and by Bolley (1) in 1910. All of these writers report an increased germination when the exposure was of short duration.

Mechanical abrasion seems for obvious reasons to have claimed the greater part of the attention of investigators. In 1890 Kuntze and Huss (8), in Germany, increased the germination of various seeds more than 70 percent by means of a scratching machine. Nilsson (10) of the Experiment Station at Savalof, designed a machine called the "preparator". This was based on the principal of throwing the seeds against a rough, rapidly revolving disk. Carrithers (3) in 1911, and Hume and Garver (5) in 1912, used machines built on the same principal and obtained increased germination. Rose (11) in 1915, by blowing the seeds against a "bank of needles", secured a decided increase in germination. Harrington (4) 1915, reported that the action of the clover huller on hard seeds of clover increased their permeability and germination. He also found that a larger percent of the seeds germinated in the spring than in the fall. Hand-hulled clover seed showed a very high percent of hard seeds. While treating the seeds of the sugar beet to remove the rough outer hull, Kuhle (7) found an increased germination.

The sulphuric acid treatment was first recommended in 1896-97, by Rostrup (12). Todardo (13) used this method in 1901,

Jarzymouski (6) in 1905, Bolley (1) in 1910, and Love and Leighty (9) in 1912. By treatment with concentrated acid for longer or shorter periods, a very marked increase in germination was noted.

Other chemicals have been tried but only the lower alcohols have been effective, (Verschaffelt (14) 1912).

The present work is an attempt to solve this important problem by means at once simple and effective.

The seeds used in these experiments were secured from Vaughan's Seed Store, the Bureau of Plant Industry, U. S. Department of Agriculture, the Agronomy Department and the Division of Floriculture of the University of Illinois. To all these the writer wishes to express his appreciation.

II Germination of the Seed.

The seeds were germinated in soil and on Plaster of Paris blocks. These blocks were six inches square by one inch thick and were grooved each way by ten parallel grooves, making 100 intersections into which the seeds were placed. These blocks, after being filled with seeds, were labeled and placed in blackened galvanized-iron pans, sixteen inches in diameter and five inches deep, containing one-half inch of water. A second similar pan was then inverted over the first one to prevent evaporation and keep the air about the seeds saturated. The blocks readily absorbed water and thus kept the seeds moist at all times. In order to hasten germination, the larger seeds, such as the canna, mallow, and cassia, were soaked in water for 24 hours or longer before placing them on the blocks. The germination pans were kept in a greenhouse automatically regulated to 70° F.

To compare the results of the germination on the blocks with possible field conditions, some of the seeds were also germinated in soil. For this purpose shallow boxes, or "flats", 12 by 24 inches by 2-1/2 inches deep, were used. The soil was sifted, placed in the flats, leveled, firmed, and watered. The seeds were then spaced evenly, in rows of 20 each, and labeled. A light covering of soil was sifted over the seeds, and the flats covered with a pane of glass to prevent excessive evaporation. The flat was then placed in a warm greenhouse.

After the seeds began to germinate a record was kept of the number sprouted each day.

In all of these experiments three sets of tests were made; first, an untreated check; second, a check in which the seeds were slightly cut with a knife to insure permeability, thus giving their true viability; and third, seeds treated by one or another of the methods to be described.

III Series I - Acid Treatment.

Canna seeds were used in the first experiments. These seeds were chosen as a type of the hardest seed coats among ornamental plants, in which the writer is especially interested. In practice, these seeds are frozen or cut to germinate them. Only sound seeds were selected for the experiments.

Sulphuric acid was the first agent used. After the acid had acted on the seeds for the required time, it was poured off and the seeds washed in water. They were then placed on the blocks to germinate. The length of these tests was about seven days.

Experiment 1

Acid 10.9 N., 26.68 N., 46.4 N., and 64.26 N., acting for 1/4, 1/2, 3/4, 1, 1-1/4, 1-1/2, 2, and 14 hours for each concentration had no visible effect on the seeds. That the seeds were not injured is shown by the fact that they were later germinated by another method.

When acid 84.2 N. was used on the seeds for 14 hours, 73 percent germinated; but for exposures from 1/4 to 2 hours, none germinated. The seeds of the 14-hour lot which had not germinated at the end of seven days were very velvety and could easily be distinguished from the treated but ungerminated seeds of the other lots. It is interesting to note here that these seeds had numerous small pits, showing an unequal action of the acid. From these experiments it was evident that a longer period of action was probably necessary in order to secure a larger percent of germination. Accordingly, the action of the 84.2 N. acid was continued for a longer period and with the following results:

Time of exposure hours	Percent germinated after 7 days on blocks
18	86
19	75
20-1/2	50
23-1/2	33

The above shows that for acid of this concentration the optimum exposure is somewhere between 14 and 19 hours.

Concentrated Sulphuric Acid sp. gr. 1.80.

Time of exposure hours	Percent germinated after 7 days on blocks
none	00
1/4 to 1-3/4	00
2	20
15	88
18	83
19	50
20-1/2	100
23-1/2	66
46	00

Some of the seeds had the entire seed coat eaten off by the acid while the coats of others were eaten thru in spots. From the above table it is easily seen that there was some variation in the results; but as these tests were discontinued, it was thot unnecessary to repeat the experiment. These tests show that a decided increase in germination can be obtained by the use of sulphuric acid of the higher concentrations, applied from 14 to 18 hours. The acid injures the embryo and prevents germination when the seeds are exposed to its action for more than 24 hours. One of the most remarkable results of this form of treatment is the large percent of germination in the first three or four days, i.e. an apparent stimulating effect. This number is greater than in seeds with the seed coats cut.

Time of exposure	Percent germinated after 10 days on blocks
none	0
Coat cut on micropyle end	50

Coat cut on opposite end	100
Coat cut on both ends	100

This table shows that no germination occurred in the untreated seeds, and 100 percent germinated in the experiment where the coats were cut without injuring the embryo.

Sulphuric acid of the higher concentrations may be used to advantage for the germination of the seeds of the canna when applied for 16 hours. No advantage is gained by treating longer than 20 hours. The untreated seed of the canna placed under favorable conditions of temperature and moisture failed to germinate in soil in six months.

Because of the difficulty of properly regulating the concentration and time of action of the sulphuric acid in relation to the varying composition or thickness of the seed coats and the impracticability of treating, washing, and drying in large quantities, it was thot desirable to try other methods. With this in view, a number of solutions were employed having a tendency to dissolve or soften the cellulose with the hope of finding one that would prove more satisfactory.

Experiment 2

Zinc chloride and _Eau de Javelle_ were tried, but gave only negative results. The zinc chloride was used in concentrations of 25, 50, 75, and 100 percent normal for 3, 24, 48, and 96 hours, then washed off and the seeds were placed on the blocks to germinate. After six days, none of these seeds had swelled, and still later were as hard as when first placed in the solution. The _Eau de Javelle_ was made according to the standard formula.

Love and Leighty (9) in their bulletin on the "Germination of Seed as Affected by Sulphuric Acid Treatment", state that "Experiments were instituted in which the seed coat was pricked with a needle, or burned with a hot needle. It was found that when the seed coat was thus broken, immediate germination was secured." This statement led the writer to believe that an effective process might be obtained by bringing the seed into contact with a hot metal plate, the idea being that the seed coat could be so changed by contact with a hot plate of carefully regulated temperature that a ready germination would be secured. The following experiments were begun to determine its feasibility and practicability.

IV Series II - Heat Treatment.

Experiment 1

The first experiment was made using the canna seed on an asbestos mat placed over a lighted Bunsen burner. The seeds were left on the hot mat for 5 and 10 seconds, and were germinated on blocks, as previously described, and records taken. At the end of five days 56 percent of those treated for 5 seconds had germinated, and 70 percent of those treated for 10 seconds. It was noted that seeds in contact with the heated mat for three or more seconds began to "jump" and "snap", and when removed and examined showed small blisters or raised places. The number of these blisters increased as the seeds remained longer on the mat. When the seeds thus treated were placed on the blocks, the water entered thru these blisters. In 24 to 48 hours after this, the entire seed coat had swelled and in many instances could be removed from the

seed. Removing the seed coat seemed to retard germination.

Experiment 2

An attempt was next made to determine, if possible, the temperature necessary to burn or blister the seed coat of the canna. A No. 9 copper wire was fastened to a ring stand over the flame of a Bunsen burner, canna seeds were then held in contact with the wire at definite distances from the flame and for varying periods of time.

Next to the flame:

Time of exposure seconds	Percent germinated
5	100
10	50
15	25
20	0

At 10 cm. from the flame:

Time of exposure seconds	Percent germinated
5	25
10	25
15	25
20	25

At 20 and 30 cm. from the flame none of the seeds swelled or germinated. Since good results were obtained only when the seeds were held close to the flame, no definite conclusions could be drawn as to the temperature required.

Experiment 3

In order to test the resistance of the seeds to higher

temperature still farther, some were held in the hottest part of the flame of the Bunsen burner for periods as follows:

Time of exposure seconds	Percent germinated after 9 days on blocks
3	25
5	25
7	50
9	75

From this table it will be seen that the seeds of the canna can be exposed to a considerable amount of heat and still germinate.

Experiment 4

A piece of sheet iron was next used. This was placed on a tripod over the flame of a Bunsen burner, and when the iron was at a dull red heat the seeds were held on by means of forceps for the following periods:

Time of exposure seconds	Percent germinated after 7 days on blocks
3	50
4	75
5	60
10	75
about 11, or till popped	85

In all of the methods used thus far the seeds have received individual treatment. A quicker and more practical method, however, was sought. In the attempt to find this, further tests were made as follows.

Experiment 5

Two short lengths of 3/4-inch pipe were joined by means of a coupling. This pipe was then fastened to a ring stand, at an angle of 35 degrees. The collar of the pipe was heated to a bright red and about 50 seeds rolled thru the pipe. The seeds used were those that failed to germinate after the dilute sulphuric acid treatment. At the end of 7 days about 60 percent of the seeds had germinated or swelled. This method was found to be uncertain since some of the seeds passed thru the heated pipe without being blistered.

Experiment 6

A heated tin cup was next tried, the seed being placed in the cup for the time stated:

Time of exposure seconds	Percent germinated after 4 days on blocks
3	75
4	75
5	62
6	75

The results from this trial show fair results and as the seeds were not treated separately was an improvement over the others.

Experiment 7

An electric hot plate having three series of resistance coils giving a low, medium, and high temperature was next used. In order to control the length of exposure without loss of time, the seeds were brought in contact with the hot plate by suddenly inverting over the hot plate a cup containing them and brushing

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]

[illegible]

[illegible]

[illegible]

them off with the cup at the expiration of the time of treatment. No germination resulted when the seeds were treated at the lower temperature up to 15 seconds. At the medium temperature, results were obtained as follows:

Time of exposure seconds	Percent germinated after 10 days on blocks
3	10
5	0
8	0
10	40

At the highest temperature:

Time of exposure seconds	Percent germinated after 10 days on blocks
3	10
5	60
8	60
10	50
15	70

The results of this trial indicate that an electrically heated plate may be used to burn the seed coat.

Experiment 8

At this point, it was thought that the same results might be obtained by rolling the seeds over high resistance wire. Two asbestos strips with the wire wound around them were arranged to form a V-shaped trough, and mounted at an angle of 30 degrees. The electricity was turned on and the seeds rolled thru the trough and down in contact with the wire. It was found that the wires were cooled by the contact with the seed and thus failed to change the coat.

Experiment 9

An apparatus was next constructed which would automatically brush the seeds from the electric hot plate. The results of these trials follow:

Time of exposure seconds	Percent germinated after 6 days on blocks
10 or less	50
10	32
15	0
20	0

This series of experiments and all those that follow, in which the hot plate was used, were made at the highest temperature, about 400° C.

Time of exposure seconds	Percent germinated	
	on blocks after 7 days	in soil after 18 days
none	0	0
none, coats cut	62	66
3	57	54
4	85	63
6	25	25
8	60	92
10	70	83
15	77	60
	after 10 days	after 12 days
none	0	0
none, coats cut	70	60
5	50	60
10	60	50

15	80	60
20	70	80
25	60	80

Seeds soaked 24 hours in water:

		after 24 days
none	0	0
none, coats cut		60
20		46

The difference in the percent of germination of treated and untreated seeds is marked and gives convincing proof of the practicability of the high temperature method for increasing seed germination. The seeds with coats cut and thus made permeable indicate, in a rough way, the viability of the sample. The close approach of the heated seeds to this standard again indicates that the method is feasible and, in fact, superior to the hand-cut method. The occasional marked variation is due to difficulty of control of the high temperature by the above method.

The encouraging results obtained on canna seeds led me to apply the method to other seeds difficult or impossible to germinate.

Experiment 10

Melilotis alba (Hand-hulled seed)

Time of exposure seconds	Percent germinated after 4 days on blocks	
Sample No. 1:	Trial 1	Trial 2
none	55	45
1/2	0	45
1	0	10

2	0	0
3	0	0
Sample No. 2 (PB 41):		
none	10	
1	5	
2	0	
3	0	
Sample No. 7:		
none	20	
rolled over plate on a 30° incline	3	

Experiment 11

Cassia Marylandica

Time of exposure seconds	Percent germinated after 10 days on blocks	
	Old seed	New seed
none	12	0
none, coat cut	50	90
1		15
2	6	10
3		0
4	0	
6	0	

The old and new seed gave a marked difference in germination, 50% and 90% respectively.

New seed heated on one edge (individual treatment for 1/2 second) germinated 90 percent. This treatment gave results equal to cutting, showing that the heating did not kill the seeds.

Experiment 12

Pueraria

Time of exposure seconds	Percent germinated after 14 days on blocks
none	40
none, coat cut	30
1	40
3	20
5	0

Cutting and heating this seed gave no increased germination.

Experiment 13

Hibiscus Moscheutos (Marsh-mallow)

Time of exposure seconds	Percent germinated after 26 days in soil	
	Soaked 24 hours	Soaked 48 hours
none	0	0
none, coats cut	95	95
1	0	56
2		55
3	33	55
5	0	

The results above show a decided increase over the untreated lot. Treatment for longer than 3 seconds proved injurious.

Experiment 14

A small quantity of Lathyrus seeds were tested in soil with the following results:

[illegible]

[illegible]

[illegible]

[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	

[illegible]

[illegible]

[illegible]

[illegible]

	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]		[illegible]
[illegible]		
[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]

[illegible]

[illegible]

[illegible]

Time of exposure seconds	Percent germinated after 26 days in soil
none	30
none, coats cut	60
5	0

5 seconds treatment killed the seed.

The quantity of seed obtainable was so limited that no further tests could be made.

The effect of possible variation of water content of seeds in storage on the variability and resistance to treatment was next tried.

Experiment 15

Seeds of Hibiscus, Cassia and Convolvulus were treated as follows: lot 1, no previous drying; lot 2, desiccated over concentrated sulphuric acid for three days; lot 3, desiccated over concentrated sulphuric acid for three days and subsequently dried in an oven at 110° C. for 24 hours. After treatment, all seeds were soaked in water for 24 or 48 hours.

Hibiscus Moscheutos

Time of exposure seconds	Percent germinated after 9 days on blocks					
	Soaked 24 hours			Soaked 48 hours		
	lot 1	lot 2	lot 3	lot 1	lot 2	lot 3
none	0	0	60	10	0	50
none, coat cut	100	100	100	90	10	50
1	50	50	70	60	70	60
2	0	40	20	0	20	20
3	0	0	0	0	0	10

Since soaking in water for a period of 48 hours shows injury, this treatment need not be farther discussed. The results in lot 3 are marked and indicate the beneficial effect of previous drying. As the simple drying at 110° C. increased the percent of germination from 0 to 60 it was thot worth while to repeat this portion of the experiment for verification. The following results were obtained:

Seeds dried at 110° C. and soaked in water for 24 hours gave 65 percent germination, those soaked 72 hours, 60 percent.

The seeds of Convolvulus when dried showed marked injury.

Drying at 110° C. for 24 hours injured the seeds of Cassia and reduced the percent of germination. Soaking in water for 48 hours before planting also caused injury. Seeds treated on the hot plate did not germinate, regardless of the previous treatment. The cut but untreated seeds and those desiccated three days over H_2SO_4 and then cut, gave from 90 to 100 percent germination in 3 days.

Seeds of the common garden asparagus did not respond to this treatment.

These trials indicate that drying is an aid to the germination of the seed of the mallow, but not to that of Convolvulus, Cassia and asparagus.

Experiment 16

Seeds of Melilotus alba, Sample No. 1, treated by rolling over a hot plate inclined at an angle of 35°:

Time of exposure	Percent germinated after 4 days on blocks
none	10
none, coats cut	100
rolled over plate once	0
" " " twice	20

Seeds of Melilotus alba when held for an instant in contact with a hot plate gave 92 percent germination.

This latter treatment shows that germination of sweet clover is possible by this method.

To test the feasibility of the application of this treatment to larger quantities of seed, the method was modified as follows: The apparatus consisted of an old-style photographic print burnisher. One of the two hollow revolving rollers was coated with asbestos paper to serve as a cushion to prevent the seeds from being crushed. The other roller, heated by means of a blast lamp, was supplied with a scraper that removed the seeds brought in contact with the hot surface. The seeds were fed from a hopper and dropped into a receptacle after contact. By altering the rate of revolution, it was possible to regulate the time of contact to the heated surface. When the rolls were revolved rapidly the time of contact was approximately one-half second, the slower rate doubled the time of contact.

Experiment 17

Melilotus alba

Time of exposure seconds	Percent germinated after 7 days on blocks		
	Sample No. 1	Sample No. 5	Sample No. 6
none	20	8	66

1/2	98	87	97
1	97	78	93

Doubling the time of exposure reduced germination slightly.

Experiment 18

Melilotus alba

Time of exposure seconds	Percent germinated after 7 days on blocks Sample No. 1	Sample No. 5	Sample No. 6
Trial one			
1/2	57	25	70
1/2 repeated twice	77	26	47
1/2 repeated three times	83	46	66
Trial two			
none		12	42
1/2		55	90
1/2 repeated twice		77	90
1/2 " three times		97	93

In trial two, the seed coats were not burned but appeared "glazed". Passing the seed thru the rollers two or three times increased the percent of germination. The treatment was repeated, rather than the time of contact prolonged, to guard against injury thru high temperature on the embryo.

Experiment 19

Melilotus alba

Time of exposure seconds	Percent germinated after 10 days on blocks	in soil
Sample No. 1:		
none	8	8
none, coat cut	100	97
1/2	96	97
Sample No. 5:		
none	10	10
none, coat cut	99	98
1/2	94	92
Sample No. 6:		
none	73	
none, coat cut	98	
1/2	75	

When sample No. 6 was treated, the roll was about 600°C. and the subsequent germination very uneven and slower than the untreated seed. It is clear that this temperature is too high for practical results. This is the only case in which treatment by this process injured the seed. Owing to the limited time, this test was not repeated. For germination of sample No. 1 in soil see Plate I, for sample No. 5 see Plate II.

Experiment 20

Hibiscus

Time of exposure seconds	Percent germinated after 10 days on blocks	in soil
none	42	
none, coat cut	92	
1	91	

Cassia

Time of exposure seconds	Percent germinated after 10 days on blocks	in soil
none	3	1
none, coat cut	97	82
1	94	92

For germination of Cassia in soil, see Plate III.

V Summary and Conclusions.

The seeds of the canna can be successfully germinated within ten days by subjecting them to the action of sulphuric acid of the higher concentrations for from 14 to 16 hours, and then washing. Treatment for over 20 hours injures the seed.

The sulphuric acid treatment is not desirable because of the length of time required for the operation and the necessity of drying the seeds if they are to be stored or shipped.

Seeds with coats impermeable to water may be made permeable and, consequently, to germinate more readily if treated to high temperature for a period sufficiently long to alter the impermeable coat.

The temperature must be sufficiently high and the time of contact so controlled that the seed coat is altered without injury to the embryo by the high temperature.

The method most promising for the application of time and temperature is that of hot rollers controlled in temperature and rate of revolution.

Aside from transforming the coats from impermeable to permeable, the application of high temperatures for a short period of time seems to increase and hasten germination.

Drying seeds previous to the application of high temperatures is beneficial in some cases, but not in others.

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VII Explanation of Plates.

Plate I.- Melilotus alba Sample No. 1.
- A. Seed coat cut.
- B. No treatment.
- C. Treated 1/2 second on hot roller.

Plate II.- Melilotus alba Sample No. 2.
- A. Seed coat cut.
- B. No treatment.
- C. Treated 1/2 second on hot roller.

Plate III.- Cassia Marylandica.
- A. Seed coat cut.
- B. No treatment.
- C. Treated 1/2 second on hot roller.

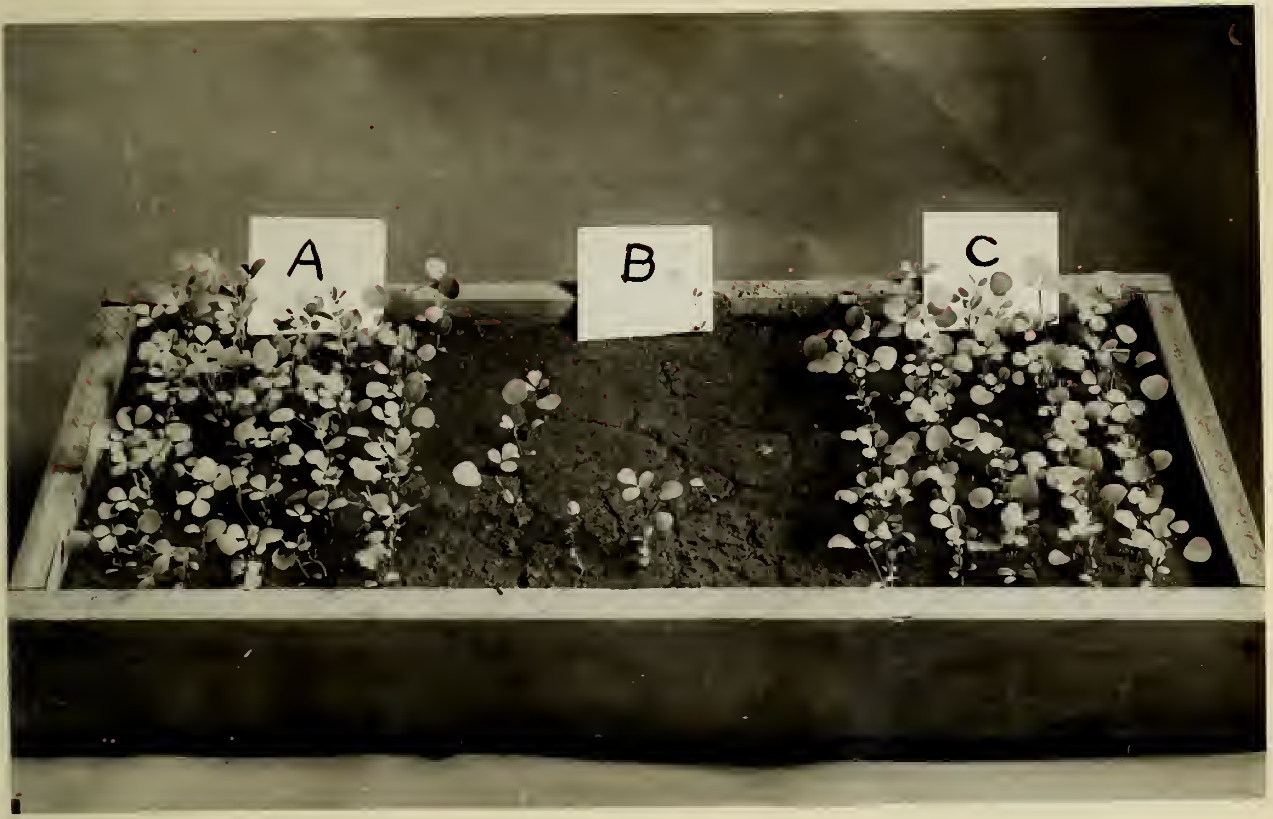

Plate I

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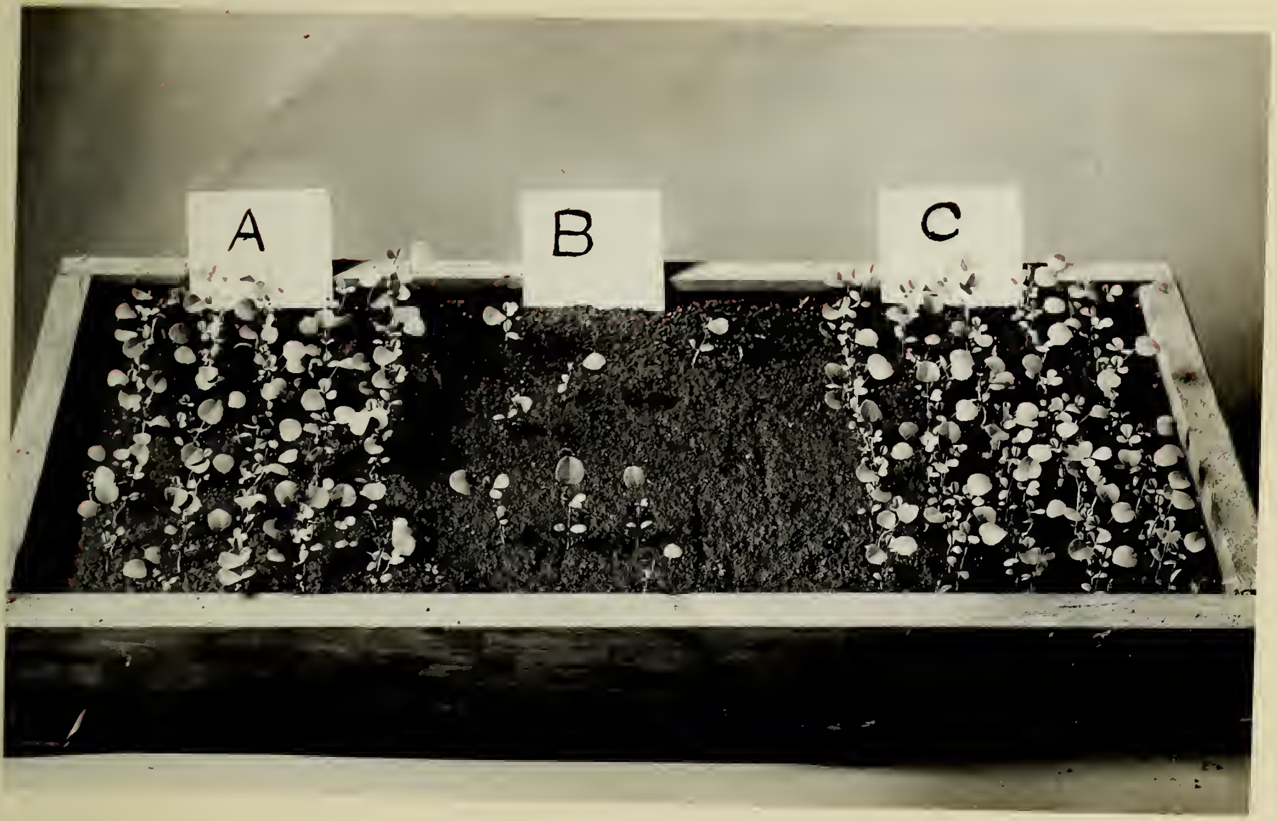

Plate II

LIBRARY
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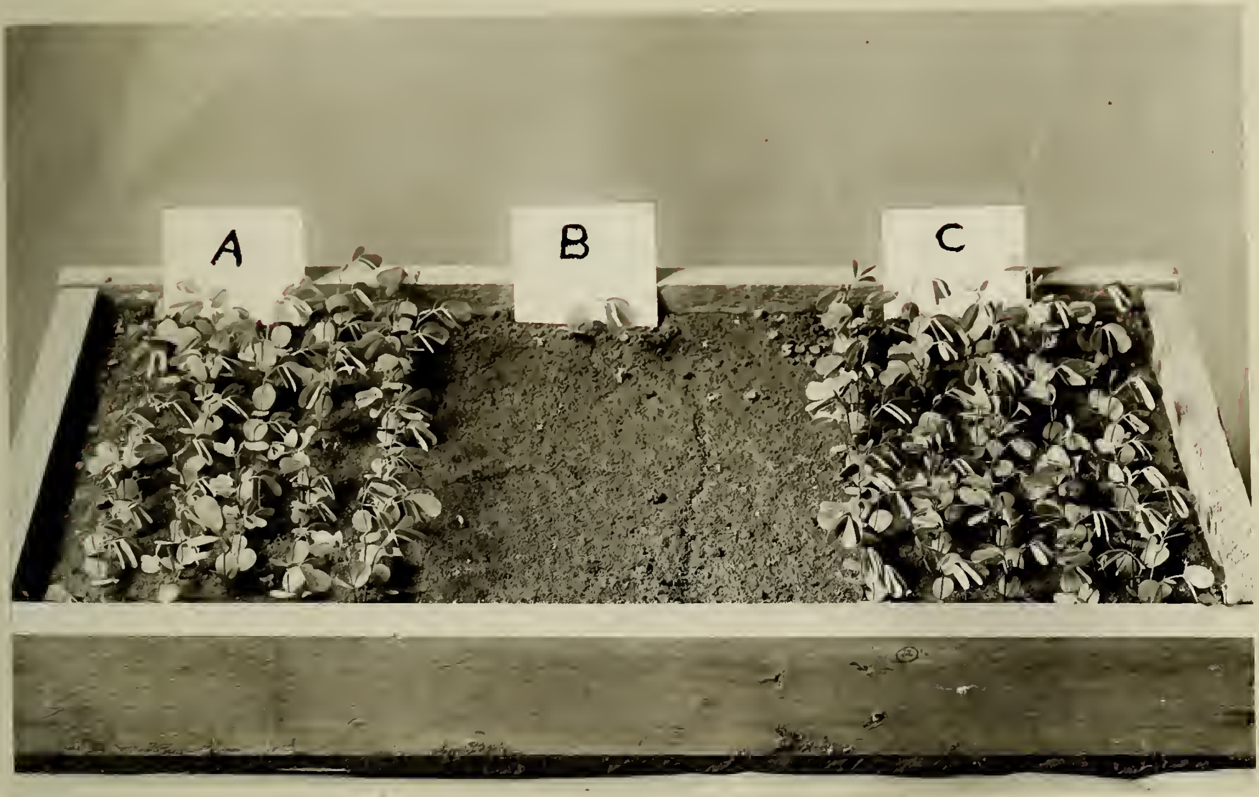

Plate III

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